THE TWO CONFORMATIONS OF TRH IN SOLUTION

J. VIČAR[++], E. ABILLON[+], F. TOMA, F. PIRIOU, K. LINTNER, K. BLÁHA*, P. FROMAGEOT and S. FERMANDJIAN

Service de Biochimie, CEN Saclay, [+]Service de Biophysique, Dépt. de Biologie, CEN Saclay, BP no. 2 91190 Gif sur Yvette, France, [++]Institute of Chemistry, Medical Faculty, Palacký University, 775 15 Olomouc, and **Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague, Czechoslovakia*

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1. Introduction

This work deals with two aspects of the thyrotropin releasing hormone (TRH) (L-pyroglutamyl–L-histidyl–L-prolinamide, ⌜Glu–His–Pro–NH_2): the conformation of the peptide backbone and the conformation of the side chains (fig.1). We proceeded by preparing compounds enriched in ^{13}C, ^{15}N and ^{2}H in order to be able to measure as many coupling constants as possible, i.e., $^{1}H-^{1}H$, $^{13}C-^{1}H$, $^{13}C-^{13}C$ and $^{15}N-^{1}H$ with the aim of analyzing them in conformational terms on the basis of appropriate Karplus type relationships [1]. In addition to the natural TRH the following labelled compounds were synthesized:

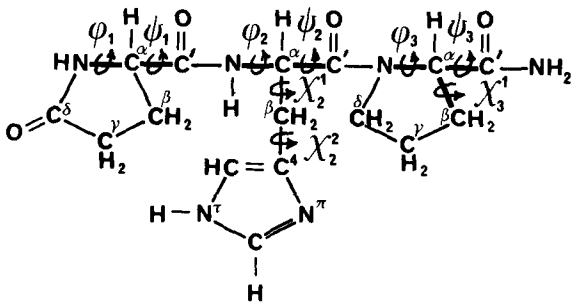

Fig.1.

Abbreviations: TRH, thyrotropin releasing hormone; NMR, nuclear magnetic resonance

Address correspondence to S. Fermandjian

[99% ^{2}H-⌜*Glu*]TRH (I), [85% ^{13}C–*His*]TRH (II), [15% ^{13}C, 95% ^{15}N *Pro*-$^{15}NH_2$]TRH (III), [85% ^{13}C-*Pro*]TRH (IV) and [15% ^{13}C, 95% ^{15}N-*His*]TRH (V). The experimental results were compared to those obtained from conformational energy calculations. We conclude that the TRH molecule may adopt two distinct conformations in solution, C_7 and 'extended', the relative abundance of which might depend on the surroundings.

2. Materials and methods

Labelled amino acids were prepared in our laboratory on a large scale from *Spirulina maxima* as in [2]. Labelled TRH was synthesized as in [3,4]. ^{1}H NMR and ^{13}C NMR spectra in the Fourier Transform mode were recorded on a CAMECA 250 MHz and a Varian XL 100-12WG spectrometer, respectively. For the semiempirical calculations we used the van der Waals parameters in [5], the charge parameters in [6], the hydrogen bond data in [7], together with a dielectric constant $\epsilon = 2$. The conformational energy maps were established using the programme 'Descartes' from [8].

3. Results and discussion

3.1. *Rotational angles* ϕ

The rotational angle ϕ_1 (in the ring) in pyroglutamic acid is comprised between 110° and 120°.

This was determined from the coupling constant $^3J_{C\alpha H-NH} = 0$ Hz [9–11]. Whereas this case hardly presents a problem, the determination of the rotational angle ϕ_2 in histidine is more difficult as the corresponding coupling constant $^3J_{C\alpha H-NH} = 7.5$ Hz allows at least for three angular solutions: $-150°$, $-90°$ and $+60°$ [9–11]. This ambiguity, due to the degeneracy of the Karplus curves was eliminated in this particular case with the help of the coupling constant $^3J_{C\Gamma_{Glu}-H^{\alpha}_{His}}$ ($\simeq 2$ Hz) obtained from compound I. The latter is tied to the rotational angle ϕ_2 in the same manner as $^3J_{C\alpha H-NH}$ (fig.1). Comparing the $^1H-^1H$ and $^{13}C-^1H$ coupling constants within their respective angular dependence curves [1] reduces the solutions for ϕ_2 to $-150°$ and $-90°$.

The rotational angle ϕ_3 in the proline residue cannot be determined from any $^3J_{C\alpha H-NH}$ coupling constants, for the ringbound nitrogen has no proton attached to it. The $^{13}C-^{13}C$ coupling, however, which can be measured on the carbonyl signal of proline in compound IV gives us access to this angle. This coupling corresponding to the contribution of $^3J_{C'-C^{\delta}}$ and $^3J_{C'-C^{\gamma}}$ yields about 0 Hz, suggesting that the corresponding torsional angles $C^{\delta}-\hat{N}-C^{\alpha}-C'$ and $C^{\gamma}-C^{\beta}\hat{-}C^{\alpha}-C'$ have mean values that can vary from $90°-110°$ each [12,13]. It follows that ϕ_3 is comprised between $-90°$ and $-70°$ and χ_1 between $10°$ and $30°$ [4].

3.2. *Rotational angles* ψ

The determination of the rotational angles $\psi_{1,2,3}$ which are inaccessible by $^1H-^1H$ couplings, was carried out with the help of $^3J_{H_i^{\alpha}-^{15}N_{i+1}}$ type coupling constants. They were obtained from proton magnetic resonances of compounds III and V, and found to be about 0 Hz for which the appropriate angular dependence curves in [1,14] yield the 4 possible solutions for $\psi_{1,2,3}$: $\simeq 165°$, $\simeq 75°$, $\simeq 15°$ and $\simeq -135°$. Although some uncertainty remains with respect to the actual values, this is the first time that one can approximate the values of ψ angles of all residues of a molecule.

3.3. *Side chain organization of histidine*

The fractions of the most stable, staggered rotamers of the histidine side chain have been proposed from proton magnetic resonance data [9]. In our study, the fraction of rotamer I is determined unequivocally from the $^3J_{^{13}C'-^{13}C^4}$ coupling constant measured in the ^{13}C spectrum of compound II. This fraction is perfectly identical to one of the rotamer fractions obtained from $^1H-^1H$ couplings (table 1). This approach therefore makes it possible to eliminate the ambiguity haunting the assignment of rotamers I and II from proton NMR spectra [15]. The stability of the coupling constants $^3J_{C^{\alpha}H-C^{\beta}H,H'}$ and $^3J_{C'-C^4}$ during pH titration reflects the stability of the mean spatial organization of the side chain with respect to its ionizable group.

3.4. *Conformational energy calculations*

Previous calculations on the TRH molecule have shown that the pyroglutamic residue can relatively freely adopt a large number of spatial orientations, notably in the zone $-60° \leqslant \psi_1 \leqslant 180°$ without interfering with the rest of the molecule [16,17]. Consequently we have chosen the value of about $15°$ for ψ_1 which among the NMR data is the closest one

Table 1

Compounds	Coupling constants ^3J (Hz)		Rotamers[a]			Conditions
			I	II	III	
TRH	$C^{\alpha}H-C^{\beta}H_2$	8.2 and 5.9	0.48	0.28	0.24	$^2H_3O^+$[b]
	$C^{\alpha}H-C^{\beta}H_2$	8.4 and 6.4	0.49	0.32	0.19	^2H_2O[b]
II	$C'-C^4$	3.0	0.46			$^2H_3O^+$
	$C'-C^4$	3.0	0.46			^2H_2O

[a] χ_2^1 angles in the rotamers are: I = $-60°$, II = $180°$, III = $60°$
[b] [9]

to the $-20°$ determined by Raman spectroscopy [10]; and we have particularly concentrated our calculations on the influence of the proline residue upon the histidine residue.

Conformational energy maps were established for 3 states of proline ($\psi_3 = 160°$, $80°$ and $30°$) and for 12 positions of the histidine side chain ($\chi_2^1 = -60°$, $180°$ and $60°$; $\chi_2^2 = -90°$, $0°$, $90°$ and $180°$) [8]. The minima obtained were centered at $\psi_2 = 90°$, $120°$ and $150°$, at $-60° \leqslant \phi_2 \leqslant -150°$ and at $\psi_3 = 80°$ and $150°$ with the distribution of rotamers I = 49%, II = 21% and III = 30% in the histidine side chain. The good agreement that exists between these values and several experimental data allows us to reduce the number of solutions by eliminating those values that do not fit. The study by the Simplex method [18,19] of the deformation of the histidyl-prolinamide part as a consequence of the histidine side chain rotation (ψ_1 remaining fixed at $15°$) suggests the possibility of a simultaneous passage of the 'extended' form (ϕ_2, $\psi_2 = -150°$, $150°$ to $165°$; ϕ_3, $\psi_3 = -60°$ to $-90°$, $150°$ to $165°$) to the C_7 form [20] (ϕ_2, $\psi_2 = -90°$, $75°$; ϕ_3, $\psi_3 = -60°$ to $-90°$, $75°$) when χ_2^1 passes through the zone $120°$ and $180°$. For $\chi_2^1 = 180°$, the histidine residue goes through the intermediate conformation given by $\phi_2 = -90°$, $\psi_2 = 120°$.

4. Conclusion

Two mean conformations are proposed [21]:

(1) C_7: with ϕ_1, $\psi_1 = 110°$, $15°$; ϕ_2, $\psi_2 = -90°$, $75°$ to $120°$; ϕ_3, $\psi_3 = -90°$ to $-60°$, $75°$;

(2) 'Extended': with ϕ_1, $\psi_1 = 110°$, $15°$; ϕ_2, $\psi_2 = -150°$, $120°$ to $150°$; ϕ_3, $\psi_3 = -90°$ to $-60°$, $120°$ to $165°$;

where in both $\chi_2^1 = -60°$ and $\chi_2^2 = 90°$ are preferred values. They possess the following characteristics: the amide group of the pyroglutamic acid ring, the peptide hydrogen and oxygen atoms of the histidine residue, the N^π nitrogen of the imidazole ring and the hydrogen atoms of the C-terminal amide group are all found on the same side of the molecule, approximately on the same plane. Small fluctuations around the torsional angles can favour electrostatic interactions of either intramolecular type (C_7 form, between the histidinyl carbonyl group and the *trans* hydrogen of the C-terminal amide group, and between the peptide NH and the N^π nitrogen of the imidazole ring of histidine when $\chi_2^1 = -60°$, $\chi_2^2 \simeq 90°$) or of intermolecular type ('extended' conformation, head-to-tail arrangement of the molecules). The 'extended' conformation might allow the TRH molecule to establish a maximum of electrostatic interactions (implicating even the imidazole N^π nitrogen important for biological activity) with its recognition site at the membrane surface of the target cell.

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